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Epidemiologic Studies on Possible Health Effects of Intake of Pyrolyzates of Foods, with Reference to Mortality among Japanese Seventh-Day Adventists

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To elucidate the effect of intake of mutagenic and/or carcinogenic pyrolysis products of proteins and amino acids on carcinogenesis in man, we have undertaken two epidemiologic cohort studies: one concerning the possible association of broiled fish consumption with cancer and the other concerning the cancer mortality among Japanese Seventh-Day Adventists. The main findings of these studies are described.

To elucidate the effect of the mutagenic and/or carcinogenic pyrolysis products of proteins and amino acids on producing cancer in man (*1*), we have undertaken two epidemiologic cohort studies: one concerning the possible association of broiled fish consumption with cancer (*2*); and another concerning cancer mortality among the Japanese Seventh-Day Adventists, which is still in progress. The main findings of these studies will be described, although results of the second study are preliminary in nature.

Cohort Sudy on Association of Broiled Fish Intake and Cancer Death

Broiled fish is a very common dish in Japan and is considered to be one of the major sources of mutagenic pyrolyzates of proteins ingested by the Japanese. The Radiation Effects Research Foundation in Hiroshima examined the dietary habits of a group of adult persons in 1968, inquiring about the frequency of intake of six food items, including broiled fish, dried fish, milk, salted pickles, fruits, and rice, together with other personal characteristics, such as date of birth, sex, school career, smoking habit, and radiation dose from the atomic bomb. For a total of 7553 subjects, consisting of 2746 males and 4807 females, complete records were available for the above 11 personal characteristics (independent variables) as well as for vital status. Therefore, we followed this cohort, aged 50.1 years on the average, from January 1, 1968, up to the end of 1978. Deaths seen during the observation period were periodically checked by utilizing the so-called "ko-seki" system, confirming the vital status of all of the cohort members. Causes of death were classified by the International Classification of Diseases, 8th Revision.

The relative risk, that is, mortality, among those consuming broiled fish twice or more weekly per mortality among those consuming broiled fish less frequently, as calculated by the Mantel-Haenszel method (*3*), was 1.33 ($p < 0.05$) and 1.67 ($p < 0.05$) for all cancer and for stomach cancer, respectively. A similar excess mortality from liver cancer was also observed among frequent consumers of broiled fish, but with no statistical significance. Mortality rates during the observation period for cancer at all sites and cancer of the stomach were calculated by sex and age and by frequency of intake of broiled fish and examined by stepwise linear multiple regression analysis (*4*) for possible association with various variables, without considering interaction effects of the variables tested, using a linear model. It was revealed that intake of broiled fish makes the fourth largest contribution to deaths from cancer at all sites, being surpassed only by age, sex, and radiation dose (Table 1). It was also shown that intake of broiled fish makes the third largest contribution to deaths from stomach cancer.

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Table 1. Results of stepwise regression analysis of deaths from cancer at all sites and stomach cancer seen in a Radiation Effects Research Foundation cohort.[a]

	Cancer at all sites		Stomach cancer	
Order	Variable	F-value	Variable	F-value
1	Age	126.93*	Age	44.85*
2	Sex	80.22*	Sex	32.37*
3	Radiation	56.87*	Broiled fish	23.22*
4	Broiled fish	44.20*	Milk	18.21*
5	Smoking	36.25*	Fruit	14.82*
6	Salted pickle	30.70*	Radiation	12.45*
7	Rice	26.54*	Salted pickle	10.71*
8	School career	23.30*	School career	9.38*
9	Fruit	20.72*	Smoking	8.34*
10	Milk	18.65*	Rice	7.51*

[a] Data of Ikeda et al. (2).
*$p < 0.01$.

A multiple regression analysis was also made, demonstrating that intake of broiled fish is significantly and positively associated with death from cancer at all sites (Table 2). A similarly significantly positive association was also seen between the intake of broiled fish and death from stomach cancer. Other variables such as age, sex, and milk consumption were also shown to be associated with stomach cancer. Our result for milk consumption was thus contrary to the observations of Hirayama (5) and Haenszel et al. (6) but similar to those of Yanai et al. (7), who did a multivariate analysis, as we did. At present, no reasonable explanation is available for this discrepancy. Furthermore, the relative risks for frequent intake of broiled fish calculated by the multiple regression equations were 1.3 for cancer at all sites and 1.7 for stomach cancer, both being very close to the corresponding values calculated by the Mantel-Haenszel method. These relative risks derived from the multiple regression equations cannot be explained by reference to any other independent variables tested, because these variables are controlled in the analysis. All these facts suggest that the observed slight but significant excess mortality from cancer at all sites and from cancer of the stomach as seen among frequent consumers of broiled fish is probably real.

Table 2. Results of multiple regression analysis of deaths from cancer at all sites and stomach cancer seen in a Radiation Effects Research Foundation cohort.[a]

	Regression coefficient × 10^4 (standard error × 10^4)			
Variable	Cancer at all sites		Cancer of stomach	
Broiled fish	97*	(38)	47*	(22)
Milk	5	(23)	27*	(13)
Salted pickle	−47	(31)	9	(18)
Fruit	−12	(30)	−19	(17)
Rice	−56	(42)	3	(24)
School career	−23	(31)	−5	(18)
Smoking	73*	(36)	4	(21)
Radiation dose	0.48+	(0.16)	0.07	(0.09)
Age at interview	16+	(2)	6+	(1)
Sex	161+	(55)	92+	(32)
Constant	−576		−329	
R^2 (%)[b]	2.41		0.99	
No. of subjects	7553		7553	
No. of deaths	244		79	

[a] Data of Ikeda et al. (2).
[b] R = multiple correlation coefficient.
*$p < 0.05$.
+$p < 0.01$.

Cancer Mortality among Japanese Seventh-Day Adventists

Seventh-Day Adventists are ovo-lacto-vegetarians (8,9). They take milk and eggs but most of them abstain from meat and fish, including broiled fish. Furthermore, virtually all of them do not smoke or drink alcoholic beverages and most of them do not drink even coffee and tea. Therefore, their overall exposures to mutagens from foods, drink, and other sources are expected to be much less than that of the general population, although no quantitative data to prove this are available yet. They seem to be unique and valuable subjects for epidemiological investigation to clarify the possible role of dietary factors and other lifestyle variables. Therefore, we became interested in the mortality from cancer among the Japanese Adventists. Unfortunately, not many Adventists (approximately 10,000) live in Japan. Thus, the size of population is limiting. Furthermore, the church records are not complete from an epidemiological point of view, in that about 15% of them do not specify either date of birth, sex, or date of baptism. After eliminating those subjects with missing information, we identified 7693 church members as Adventists who had been converted at least 5 years before January 1, 1975 or had had their fifth anniversary of conversion during the observation period (the 6 year period from January 1, 1975 to January 1, 1981). Vital status as of January 1, 1981, was determined for each subject by examining the records preserved at the central office of the church or by inquiring at the local offices.

If we were unable to confirm vital status by these methods, we made inquiries to municipal offices at their permanent addresses, utilizing the "koseki" system. As shown in Table 3, however, 951 (12.4%) of the cohort members could not be confirmed for their vital status by any of these means. This was almost entirely due to clerical failures to record the permanent address. These 951 members were eliminated, and the remaining 6742 subjects were followed. The number of deaths observed

Table 3. Vital status of Seventh-Day Adventists.[a]

Sex	Alive	Dead	Unknown	Total
Male	1944	95	315	2354
Female	4506	197	636	5339
Total	6450	292	951	7693

[a] Number of subjects: 7693 − 951 = 6742

among this group during the observation period (but only after the fifth anniversary of conversion) was then compared with the corresponding expected number of deaths, calculated by multiplying age-sex-specific person-years at risk with age-, sex-, and cause-specific mortality rates for the Japanese population in 1975.

As shown in Table 4, the O/E ratio for all causes of death was 0.77 for males and 0.74 for females, which were significantly less than unity. Since, as mentioned, those whose vital status was unknown were eliminated from the present calculation, such elimination might be associated with the observed reduction in overall death. This does not seem probable, however, because: (1) the present cohort is a specific religious one, consisting of probably the most religious persons among the Japanese, and any deaths would be notified to the church without fail; (2) our failure to confirm vital status was, as mentioned, almost exclusively due to clerical oversight in recording the permanent address, and such oversight is unlikely to associated with the health status or survival of the subjects; and (3) those subjects eliminated from the analysis because of unknown vital status and those who could be followed were comparable in sex and age composition. Thus, the overall reduced mortality observed seems real. For cancer at all sites, a highly significantly lowered mortality was observed in males, and the mortality for females was also lower than expected but to a lesser degree and without statistical significance. For cancer of the stomach, a significantly reduced mortality was observed for both males and females. For cancers of the esophagus, intestine and colon, rectum, liver, pancreas, and lung, and for leukemia, statistically significant deviations in mortality were not observed for either males or females. Mortality from cancer of the respiratory system was found to be significantly reduced only for males.

For diseases other than cancer, a notably reduced mortality was observed for cerebrovascular diseases in males and females. The O/E ratio for ischemic heart disease was 1.0 in males but was significantly less than unity in females. No statistically significant reductions or elevations in mortality were observed for tuberculosis and diabetes in males or females.

All the above findings obtained from the cohort studies seem to suggest that mortality from cancer at all sites and from cancer of the stomach might be associated with the intake of pyrolysis products of proteins and amino acids. However, the evidence is still weak and vague, and no conclusive remarks can be made. Further investigations may provide further support for our findings.

The question as to whether or not the pyrolysis products of foods are carcinogenic to man will be very hard to answer by epidemiological studies, mainly because cooking is a universal practice, and all men are exposed to mutagenic pyrolyzates of foods to a substantial degree. Thus, it is probably not possible to find persons who have extremely low levels of exposure to them. Also, estimation of an individual's exposure to single mutagenic pyrolyzates of food components is not yet possible. Of these two difficulties, the former seems impossible to solve in the future, because we shall continue cooking of foods. To make matters more difficult, man is exposed to complex environmental mutagens other than mutagenic food pyrolysates. It will be extremely difficult to demonstrate unequivocally by simple epidemiologic approaches any specific health effects which might be caused by intake of such pyrolyzates. In this respect, Seventh-Day Adventists seem to be an extremely valuable study population, because as already mentioned, their exposure to environmental mutagens is much simpler than that of the general popu-

Table 4. Observed and expected number of deaths and 0/E ratio by sex and cause of death among Japanese Seventh-Day Adventists.

	Male			Female		
Cause of death	Observed	Expected	O/E	Observed	Expected	O/E
All causes	95	122.7	0.77*	197	267.2	0.74^{+}
All cancer	7	23.6	0.30^{+}	36	46.0	0.78
Esophagus	0	0.9	0	0	1.2	0
Stomach	3	9.5	0.32*	4	15.3	0.26^{+}
Intestine and colon	1	0.9	1.11	1	2.4	0.41
Rectum	0	1.0	0	3	2.2	1.36
Liver	0	2.1	0	3	3.0	1.00
Pancreas	0	1.0	0	3	1.9	1.54
Respiratory system	0	3.8	0*	4	3.9	1.04
Lung	0	3.2	0	4	3.2	1.25
Leukemia	0	0.5	0	0	1.0	0
Breast				2	2.5	0.80
Cervix				1	1.2	0.84
All tuberculosis	4	2.4	1.67	3	2.5	1.21
Diabetes	2	1.4	1.43	1	3.7	0.27
Cerebrovascular disease	12	31.7	0.38^{+}	32	74.1	0.43^{+}
Ischemic heart disease	18	18.1	1.00	29	43.4	0.67*

*$p < 0.05$.
$^{+}p < 0.01$.

Table 5. Comparison of Seventh-Day Adventists with general population in regard to exposure to environmental mutagens.

Population	Tobacco	Alcohol	Coffee	Tea	Meat	Fish	Group
General	+	+	+	+	+	+	A
population	−	−	−	−	+	+	B
SDA	−	−	+	+	+	+	C
	−	−	−	−	+	+	D
	−	−	+	+	−	−	E
	−	−	−	−	−	−	F

lation, allowing us a more refined and precise analysis of the risk of diseases which might be associated with different dietary habits. As shown in Table 5, if we could compare the risk of diseases between group B and group F consisting of the most devoted Adventists who compose about a half of the Adventists in the United States (*5,6*), or between group D which consists of Adventists not strictly abstaining from meat, poultry, and fish, and group F, it might be possible to elucidate the possible effects of the intake of mutagenic pyrolyzates of foods. Unfortunately, such an epidemiologic study cannot readily be done in Japan because the number of Adventists is limited there, while it may be possible in the United States.

If the second difficulty could be overcome in the future, the epidemiologic elucidation of the present question will become far more precise and efficient. In order to clarify epidemiologically the possible health effects of the intake of a specific mutagenic compound formed by cooking, we, of course, have to know the individual exposure to such compound. This is very important in view of the fact that quite a few different mutagens have been isolated from food pyrolyzates. Good cooperation with chemical analysts seems particularly important for the epidemiologic study.

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